ANTIFUNGAL AND ANTIOXIDANT PEPTIDES FROM OIL PALM MESOCARPS

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ABSTRACT

Phytochemicals and bioactive peptides have been discovered in oil palm organs such as kernel and leaf, but there is no documented study in isolating peptides with bioactivities from fruit mesocarps. In this work, we aimed to determine the bioactivity of the peptides derived from hydrolysis of oil palm fruit mesocarps. The fruit mesocarps were subjected to enzyme hydrolysis using trypsin and pepsin, followed by separation of these peptides through liquid chromatography-tandem mass spectrometry (LC-MS/MS) and database searching. Analysis of bioactivity from 25% and 30% fractions (trypsinised), and 10% and 30% fractions (pepsinised) showed inhibitory effect towards the growth of Ganoderma boninense. *In antioxidant assay, only non-fractionated protein hydrolysate with trypsin and pepsin showed a scavenging activity of 17.7%-22.3% for 2,2-diphenyl-1-picrylhydrazyl. We further determined the possible occurrence of antimicrobial activity* in silico *by matching the experimental peptide sequences to known bioactive peptides. Four antimicrobial peptides were found in fractionated mesocarp hydrolysates using trypsin and pepsin. These bioactive peptides had molecular masses of less than 4-5 kiloDalton (kDa) and made of 11-39 amino acid residues. A total of 23%-54% hydrophobic amino acids were found within these sequences. The results showed that the oil palm mesocarps contained peptides with various bioactivities that could be bioprospected into functional constituents with health and crop protection benefits.*

Keywords: antifungal, bioactive peptides, enzyme hydrolysis, *Ganoderma boninense*, oil palm.

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INTRODUCTION

Crude palm oil (CPO) extraction from fruit bunches in palm oil mills around Malaysia generate waste by-products in the form of solid (palm kernel shells, mesocarp fibres and empty fruit bunches-EFB) and liquid (palm oil mill effluent). In 2019, there were 452 palm oil mills in operation throughout Malaysia, capable to produce 112.91 million tonnes of fresh fruit bunches (FFB) annually (Kushairi and Mohd Din, 2020). Chavalparit (2006) reported that an average value of waste generation rate per tonne FFB from palm oil mill in Thailand were 140 kg of fibre, 60 kg of shells, 240 kg of EFB and 42 kg of decanter cake. Abas *et al*. (2011) reported that these by-products could reach 70-80 million tonnes per year. Irvan (2018) also stated that for every tonne of CPO produced, 0.90 t of EFB, 0.60 t of mesocarp fibres and 0.27 t of palm kernel shells are generated. This huge amount of waste by-products must be treated efficiently or used as biomass to reduce their undesired impacts to the environment.

Mesocarp fibres are generated from mesocarp pressed cake after the removal of nuts and pulp oil (Sabil *et al.*, 2013; Sreekala *et al.*, 1997). Together with EFB and palm kernel shells, the fruit fibres can be considered harmful waste to the surrounding environment, if released untreated. These fibres are

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harder to dispose and normally discarded in the palm oil mills as wastes or used as boiler fuel to produce steam and generating power due to their low moisture content compared to other biomass residues such as EFB. However, mesocarp fibres have low carbon content and therefore, require a remarkably high load to generate power energy, compared to coal. Mesocarp fibres also have low heating value, rendering it difficult to be utilised (Pereira *et al.*, 2020).

Bio-based products are a growing trend in industrial crops. The increasing need for these products is the driving force for the growth of functional food market. Bioactive peptides with antioxidant properties have been indicated as one of the key ingredients in health-promoting foods. The peptides work to lessen the effects of oxidative stress and lipid peroxidation caused by free radicals in human body and food products (Tadesse and Emire, 2020). A demand for less detrimental agricultural pesticides with reduced detrimental effects for crop protection is also pushing for the emergence of the peptide-based biopesticide industry. Biopesticides work similarly with chemical pesticides but not toxic and damaging to the environment. The oil palm industry is currently being threatened by *Ganoderma boninense*-induced basal stem rot disease. Biofungicides could provide an alternative to the management of this disease without the use of chemical-based control agents.

Mesocarp pressed cake and fibres are readily available raw materials for the extraction of bioactive peptides with antioxidant and antifungal properties. Through this technology, a downstream processing technology to convert mesocarp-based materials into value added natural product can be developed. Bioactive peptides represent specific sequences of amino acids that possess biological activity with several health effects and potential applications in nutraceutical industry. These peptides can be obtained from a chain of procedures, including cell lysis and protein extraction and enzymatic hydrolysis. Mesocarp fibres can have economic values as they can be used as sources of these biomaterials. Protein hydrolysates from palm kernel cake had been found to exhibit good angiotensin converting enzyme (ACE)-inhibitory activity between 22.9% and 70.9% (Zarei *et al.*, 2015), antiradical capacity (Ng *et al.*, 2013) and antibacterial activity (Tan *et al.*, 2013). It is therefore rationale to investigate if mesocarp tissues also contain other natural occurring peptides to be developed as bioactive defense against harmful environments. The current process using fruit mesocarp could be adapted to produce bioactive peptides from the abundant mesocarp pressed cake and fibres. To our best knowledge, there is no reported use of oil palm fruit mesocarp as raw material to produce protein hydrolysate, nor is there any published work on the bioactive properties of the protein hydrolysate from this important oil crop.

MATERIALS AND METHODS

Plant Materials

Ripe *Elaeis guineensis* (oil palm) fruitlets (20th week after anthesis) were used as the source of proteins for enzyme hydrolysis. The fruit mesocarps from the fruitlets were sliced to about 2 cm in length, snap frozen in liquid nitrogen and stored at -80°C.

Protein Extraction

Protein extraction was performed according to Lau *et al.* (2015). Five g of sliced fruit mesocarps were ground in 15 mL of acetone containing 10% trichloroacetic acid and 1 mM dithiothreitol. The slurry was then centrifuged at 13 000 g for 10 min at 4°C (RA-300 rotor, Kubota 7820, Kubota Corporation, Tokyo, Japan). The washing step was repeated prior to addition of 15 mL of 80% methanol containing 0.1 M ammonium acetate. The slurry was mixed and centrifuged as before. The precipitated mesocarp pellet was washed with 15 mL of 80% acetone. The mixture was centrifuged at 13 000 g for 10 min at 4°C. Pellet was re-suspended in 15 mL of extraction buffer containing 0.7 M sucrose, 1 M Tris-HCl, pH 8.3, 5 M NaCl, 50 mM DTT, and a tablet of Roche protease inhibitors. The resuspension was sonicated in ultrasonic bath for 15 min (Townson Mercer Ltd., Stretford, United Kingdom). The mixture was then sieved through two layers of Miracloth (Calbiochem, EMD Millipore Corporation, Billerica, MA, USA) to isolate the non-macerated plant materials. An equal volume of fresh 50 mM, pH 8.0 Tris-saturated phenol (15 mL) was added to the mixture, mixed, and centrifuged at 15 000 g for 15 min at 4°C. Proteins in the upper phase were precipitated by adding 5 volumes of cold ammonium acetate-saturated methanol and incubated at -20°C overnight before being centrifuged at 15 000 g for 15 min at 4°C. The protein pellet was rinsed with ammonium acetate-saturated methanol and washed twice with 10 mL of 80% acetone. The protein pellet was air-dried.

Protein Hydrolysis and Fractionation

Pellet was solubilised in 50 mM Tris-HCl, pH 8.0 (for trypsin) or 50 mM KCl, pH 2.0 (for pepsin). A 1 mg of trypsin or pepsin was added to 50 mg of proteins. The mixture was incubated for 24 hr with agitation rate of 300 rpm (Innova 42, New Brunswick Scientific Co. Inc., CT, USA). Reaction

was stopped by heating at 100°C for 10 min. The hydrolysed peptides were filtered with 10 kDa centrifugal filters (Amicon Ultra, Merck Millipore Ltd., County Cork, Ireland), purified with C18 disk and fractionated through sequential elution with 5%-80% acetonitrile.

2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Free Radical Scavenging Activity Assay

A volume of 50 mL of DPPH solution was prepared using 1.25 mg of 2,2-diphenyl-1-picrylhydrazyl and 50% methanol. An assay standard of 1 mL was prepared using 25 mg gallic acid and 5 mL of double-distilled water (ddH$_2$O). A volume of 15 µL of assay standard or sample was mixed with 975 µL DPPH solution. Absorbance at 515 nm was read every 20 s for 12 cycles (Infinite M200, Tecan, Mannedorf, Switzerland). The antioxidant activity was expressed as percentage of DPPH free radical scavenging activity (%DPPH$_{SC}$) and calculated using the Equation (1):

$$\%DPPH_{SC} = (ABS_{blank} - ABS_{sample}) / (ABS_{blank} \times 100\%) \qquad (1)$$

where ABS$_{blank}$ is the absorbance value of blank at t=195 s and ABS$_{sample}$ is the absorbance value of protein hydrolysate. Gallic acid at a concentration of 0-10 µg mL^{-1} was used as the assay standard.

Antifungal Assay

A volume of 100 µL of potato dextrose broth was added into the well of a 96-well microtiter plate. The potato dextrose broth was mixed with 10 µL of sample in the well. A plug of the *G. boninense* fungus, with a diameter of 5 mm, was added into the well. In this assay, well with a plug of the *G. boninense* fungus without the sample was used as a positive control. The well containing just the potato dextrose broth was used as a negative control. The plate was incubated in dark at 27°C. Growth was observed until day 7.

Liquid Chromatography-tandem Mass Spectrometry

Separation and spectra acquisition of the peptides was conducted with a Thermo EASY-nano liquid chromatography 1200 System, coupled to a Thermo Q Exactive Plus mass spectrometer (Thermo Scientific, MA, USA). The dried peptide digests were reconstituted in 30 µL of 0.1% formic acid and 5% acetonitrile. A digest volume of 2 µL was injected into an Acclaim PepMap 100 C18 reversed phase column (3 µm, 0.075 x 250 mm) (Thermo Scientific, MA, USA). The column was equilibrated with 0.1% formic acid (Mobile phase A) and 80% acetonitrile containing 0.1% formic acid (Mobile phase B). Gradient of 5%-35% in 90 min was applied at a flow rate of 300 nL min^{-1}. Peptide ions were generated by electrospray ionisation using a spray voltage of 1900 V. Peptide precursor survey scan with a mass ranged from m/z 310-1800 and resolving power of 70 000 was acquired. Only peptide precursors with charge state of 2-7 were chosen for tandem MS. Tandem MS conditions consisted of rapid scan rate using a resolving power of 17 500 and 0.7 m/z isolation window. Precursors were fragmented using collision induced and high-energy collision induced at normalised collision energy of 28%, respectively. Mass range scanned was from m/z 110-1800.

Data Analysis

Data acquisitions in positive mode were executed with Thermo Scientific Tune. Generated raw data was processed with Thermo Scientific Proteome Discoverer, version 2.2 (Thermo Scientific, MA, USA). Tandem mass spectra were searched with SEQUEST HT engine against antimicrobial peptide FASTA sequences obtained from the Antimicrobial Peptide Database (APD) (Wang *et al.*, 2016) to determine peptide with bioactivity. Mass tolerances for peptide and product ions were set to 20 ppm and 0.5 Da. Trypsin or pepsin with customised cleavage sites was designated as the protease with two missing cleavages was allowed. Carbamidomethylation on cysteine was set as the static modification while oxidation of methionine, deamidation of asparagine and glutamine, and acetyl N-terminal modifications were searched as dynamic modifications. All peptide spectral matches were validated using the Percolator (component of Proteome Discoverer) based on q-value at a 1% false discovery rate.

RESULTS

The process to convert the oil palm mesocarps to produce bioactive peptides comprised of a series of steps from protein extraction to fractionate the obtained protein hydrolysates using enzymatic hydrolysis (*Figure 1*). The subsequent search for other bioactive peptides involves generating the sequence of the fractionated peptides and matched them to existing antimicrobial databases to identify possible antimicrobial peptides (Odintsova *et al.*, 2009).

Antifungal Assay

Fractionated peptides were qualitatively tested for their antifungal bioactivity, specifically towards *G. boninense* using a microtiter plate-based assay. As indicated in *Figure 2*, 25% and 30% fractions of the trypsin hydrolysate showed inhibition of the fungus *G. boninense* at day 2 to day 3. Eluted fractions 10%

and 30% of the pepsin hydrolysate also demonstrated inhibition against *G. boninense* from day 2 to day 3. In this antifungal activity assay, we had monitored the inhibition behaviour until day 7 of the *G. boninense* growth. At day 7, *G. boninense* growth no longer show any inhibition by the peptide (for both trypsin and pepsinised) hydrolysates as the fungal growth reached saturation at day 7. It was postulated that the dosage of the peptide hydrolysates (10 µL) was enough to suppress the growth until day 3.

2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Free Radical Scavenging Activity Assay

Only non-fractionated mesocarp protein hydrolysates were assayed for their scavenging activity towards DPPH. *Figure 3* shows the $DPPH_{SC}$ of the trypsin and pepsin hydrolysates. Presence of peptides with scavenging activity of 22.3% was found in pepsin-treated mesocarp proteins after 3 min. Trypsin-digested mesocarp
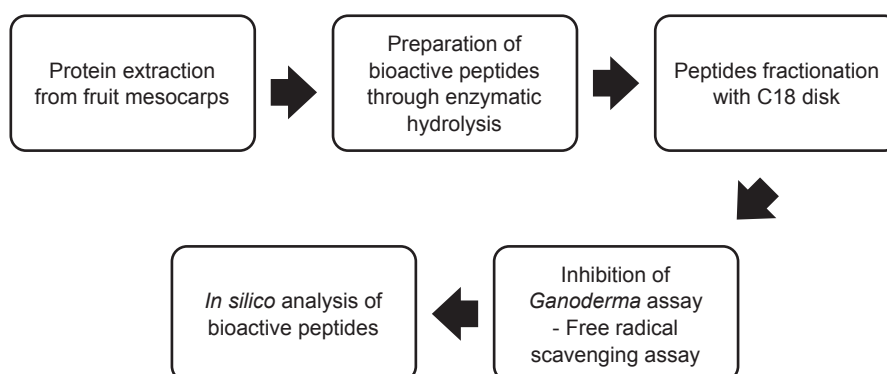

Figure 1. Overall process to generate bioactive peptides from oil palm mesocarps. The protein was extracted from the oil palm fruit mesocarps before being treated with trypsin and pepsin for enzyme hydrolysis. Then, the peptides were fractionated with C18 disk for antifungal, while the non-fractionated hydrolysates were used for antioxidant assay. The acquired peptides were further matched to the antimicrobial peptide database (APD) to determine the existence of bioactivity.
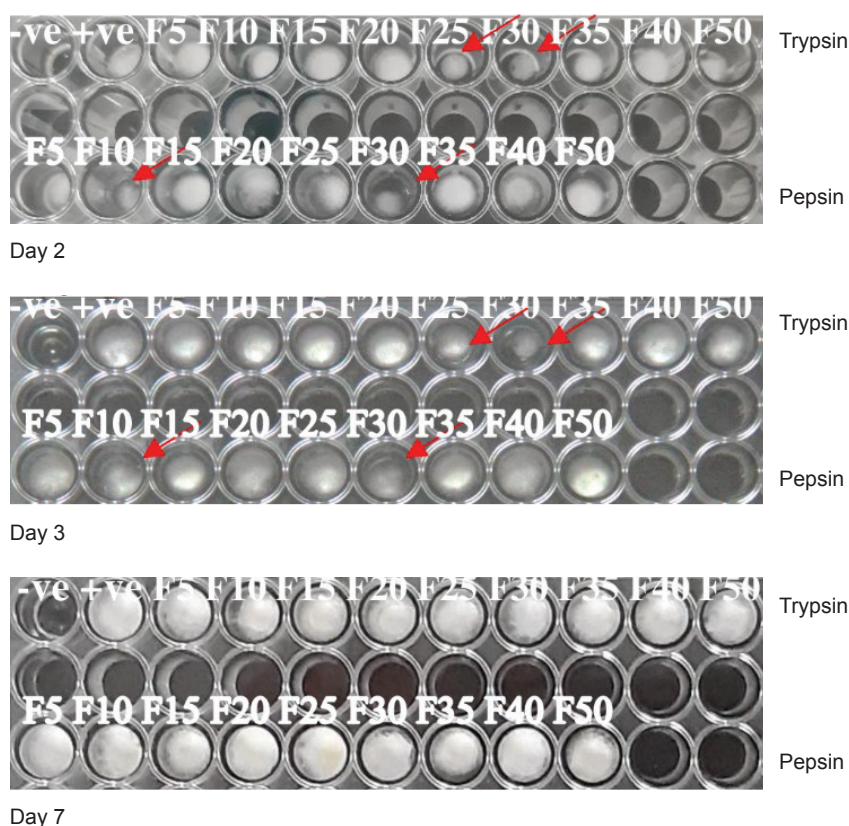

Figure 2. Antifungal bioactivity of the fractionated peptide hydrolysates towards Ganoderma boninense *until day 7 of the fungal growth. The fractionated trypsin and peptide hydrolysates (F5, F10, F15, F20, F25, F30, F35, F40 and F50) were tested for their inhibition against the* Ganoderma boninense *fungus. F25 and F30 of the trypsin hydrolysate showed inhibition at day 2 to day 3. F10 and F30 of the pepsin hydrolysate also showed inhibition from day 2 to day 3.*
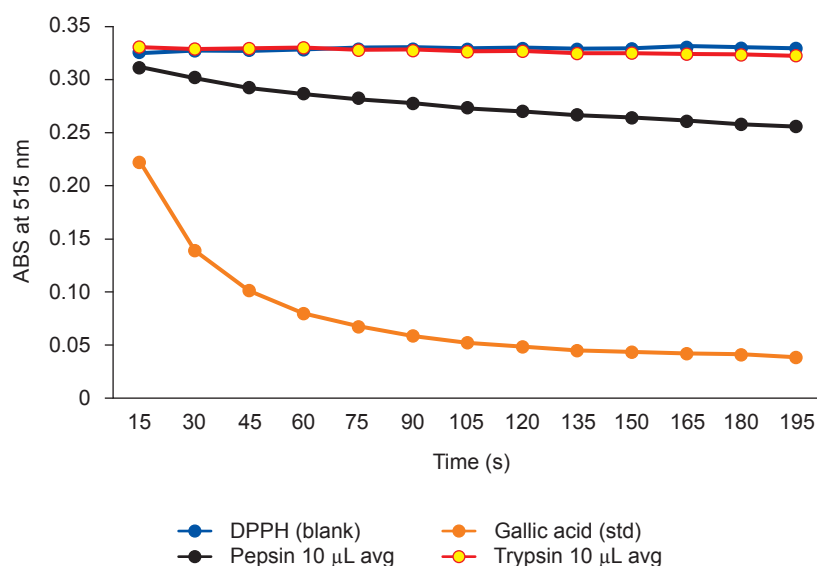
Figure 3. Free radical-scavenging activity assays of trypsin- and pepsin-treated mesocarp proteins. Gallic acid was the assay standard used and DPPH was the assay blank. Pepsin hydrolysate showed 22.3% of scavenging activity and trypsin hydrolysate exhibited a lower scavenging activity of 17.7%.

proteins showed lower $DPPH_{SC}$ of 17.7% after 3 min. Digestion with pepsin clearly exposed more amino acid residues that could act as hydrogen donor to enhance the scavenging capability of the peptides. Digestion with trypsin resulted in peptides with higher hydrophilicity that render them inaccessible to DPPH free radicals (Bamdad *et al.*, 2011). Both hydrolysates were filtered and supposed to comprise of peptides with masses of less than 10 kDa. Low mass peptides are normally more effective as antioxidants (Hong *et al.*, 2014; Liu *et al.*, 2015; Zhao *et al.*, 2007).

In Silico Identification of Peptide with Antimicrobial Bioactivity

Sequence of the fractionated peptides was determined using shotgun proteomics strategy. *Figures 4* and *5* displayed the representative peptide fragment spectra from the fractionated hydrolysates corresponding to trypsin and pepsin hydrolysis. The peptide sequences were searched against the antimicrobial database to identify peptides with bioactivities. The *in silico* analysis found four peptides with mass of less than 4 kDa (MH+) obtained from the trypsin digestion matched (82%-100%) to known antimicrobial peptides deposited in the database. Their sequences contained 11-39 amino acid residues per peptide. Three of these peptides identified to PG-K111, Peptide 5 and lactococcin Q, possess anti-gram positive and negative activities while peptide NKGCAICSIGAACLVDGPIPDFEIAGATGLFGLWG (identified to subtilosin A1) from fraction 15% has anti-gram positive property only. All these

peptides have high amount of hydrophobic amino acids (23%-54%). The increment of these hydrophobic residues explained the higher concentration of acetonitrile to elute these bioactive peptides and their solubility in lipid. This could help in enhancing the lipid inhibitory activity by facilitating the interaction between peptides and radical species (Siow and Gan, 2013). Hydrophobic amino acid could also help to exhibit higher antihypertensive potential (Cheung *et al.*, 1980; Ghribi *et al.*, 2015).

Peptide sequences identified from 5% peptide fraction of pepsin digestion were found matched to the known antimicrobial and antifungal peptides. All these peptides have mass of less than 5 kDa (MH+). All the matched peptides identified to human drosomycin-like defensin, MiAMP2c and MiAMP2d, have antifungal properties except peptide RMRRSKSGKGSGGSKGSGSKGSKGSKGS GSKGSGSKGGSRPGGGSSIAGGGSKGKGGT QTA, identified to ayu cathelicidin (aCATH) peptide in the APD. Less than 25% of the peptides are made of hydrophobic amino acids and these explained the poor retention by the hydrophobic column. The *in silico* analysis also showed that these peptides are very long, from 48-68 amino acid residues per peptide. The peptide sequences were observed to possess a high amount of repeating amino acid residues. Many reported dipeptides from plant proteins were found to exhibit *in vitro* antioxidant effects (Samaraweera *et al.*, 2011). These di- and tri-peptides have exhibited better biological activity if compared to their constituent amino acids (Kawashima *et al.*, 1979). They are also absorbed more rapidly than free amino acid (Silk *et al.*, 1980).
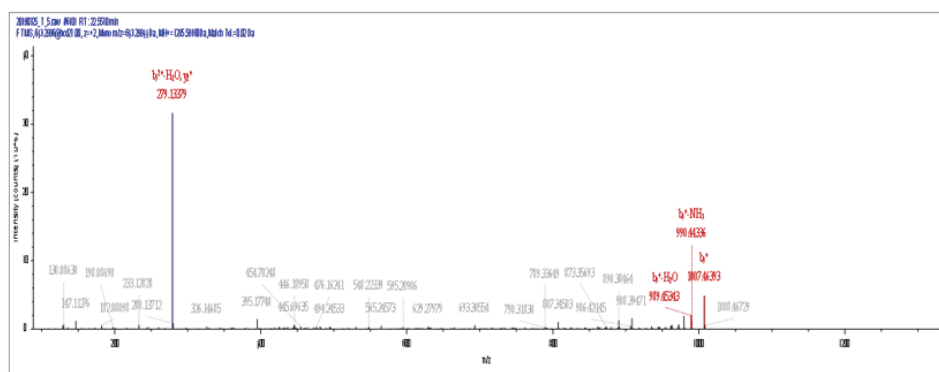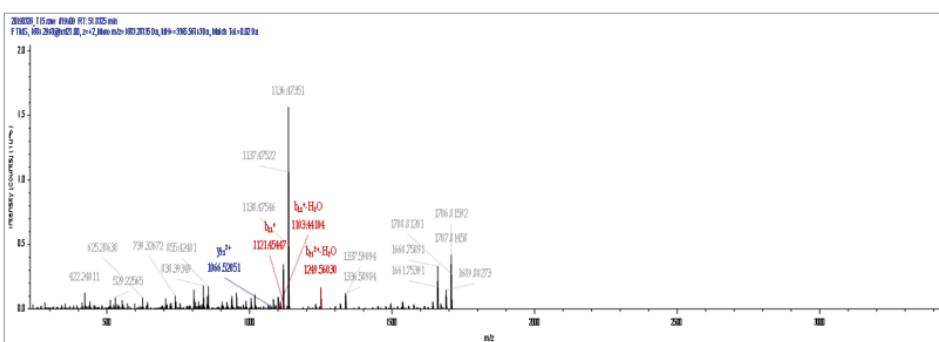
EPHPNEFVGLM (MH+: 1285.58815 Da, Fraction 5% and 20%)
36% hydrophobic residue, length: 11 amino acid
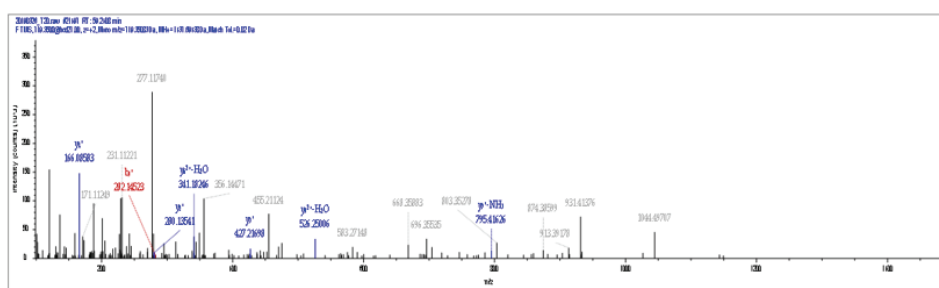
SPPSEQLGKSFNF (MH+: 1437.70087 Da, Fraction 10%)
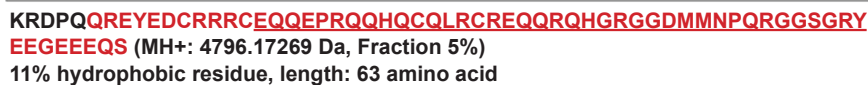23% hydrophobic residue, length: 13 amino acid
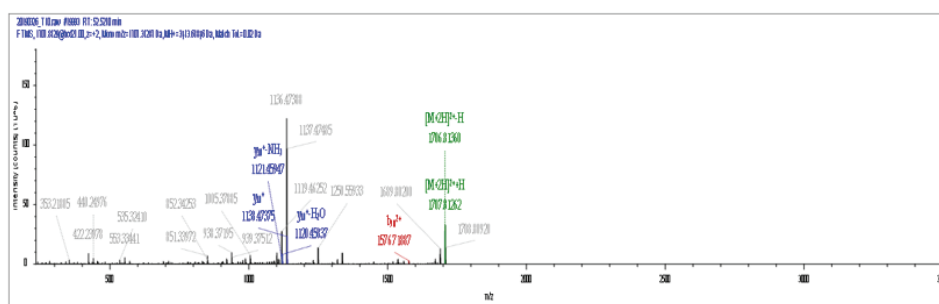
SIWGDIGQGVGKAAYWVGKAMGNMSDVNQASEINRKKKH (MH+: 3413.58890 Da, Fraction 10%)
35% hydrophobic residue, length: 39 amino acid

NK**GCAICSIGAACLVDGPIPDFEIAGATGLFGLWG** (MH+: 3365.60032 Da, Fraction 15%)
54% hydrophobic residue, length: 35 amino acid

Figure 4. Peptide spectra identified as bioactive peptide from 5%, 10%, 15% and 20% fraction of trypsin hydrolysate. The peptide sequences determined from this work were matched to known bioactive peptide sequences in Antimicrobial Peptide Database (APD). Sequence matched is in red font and underlined. The peptides were identified to PG-K111, Peptide 5, lactococcin Q and subtilosin A1.

RMRRSKSGKGS<u>GGSKGSGSKGSKGSKGSGSKGSGSKGGSRPGGGSIAGGGSKGKGGTQTA</u>
(MH+: 4211.11075 Da, Fraction 5%)
6% hydrophobic residue, length: 61 amino acid

CLAG<u>RLDKQCTCRRSQPSRRSGHEVGRPSPHCGPSRQCGCHMD</u> **(MH+: 4707.09939 Da,**
Fraction 5%)
25% hydrophobic residue, length: 43 amino acid

RQ<u>RDPQQQYEQCQKHCQRRETEPRH</u>MQTCQQRCERRYEKEKRKQQKRYEEQQREDEEKY
(MH+: 4683.18453 Da, Fraction 5%)
8% hydrophobic residue, length: 68 amino acid

KRDPQQ<u>QREYEDCRRRCEQQEPRQQHQCQLRCREQQRQHGRGGDMMNPQRGGSGRY</u>
EEGEEEQS **(MH+: 4796.17269 Da, Fraction 5%)**
11% hydrophobic residue, length: 63 amino acid

Figure 5. Peptide spectra identified as bioactive peptide from 5% fraction of pepsin hydrolysate. The peptide sequences determined from this work were matched to known bioactive peptide sequences in Antimicrobial Peptide Database (APD). Sequence matched is in red font and underlined. The peptides were identified to aCATH, human drosomycin-like defensin, MiAMP2c and MiAMP2d.

DISCUSSION

This study described the isolation of peptides with validated antifungal towards *G. boninense* and antioxidant bioactivities from oil palm fruit mesocarp. *In silico* analysis also revealed the presence of antimicrobial peptides after tryptic and peptic hydrolysis of the oil palm fruit mesocarp. There is no reported study that examined the conversion of oil palm mesocarp to generate these bioactive peptides. The nearest oil palm related materials that were used to produce peptides with bioactivity were extracted from palm kernel cake and pulp (Chee *et al.*, 2012; Ng *et al.*, 2013; Tan *et al.*, 2013; Zarei *et al.*, 2015; Zheng *et al.*, 2017).

In this work, the 25% and 30% fractions of trypsin hydrolysate were observed to suppress the growth of *G. boninense*. However, *in silico* analysis found four antimicrobial peptides in 5%-20% fractions of trypsin hydrolysate, instead. Similarly, the 10% and 30% fractions of the pepsin hydrolysate were found to suppress the fungal growth until day 3 before the effect wore out by day 7. *In silico* analysis identified four peptides with reported antifungal and antimicrobial activity from 5% fraction of the pepsin hydrolysate only. The results indicated that the peptide with antifungal activity towards *G. boninense* could be novel and has not been reported previously. Antimicrobial and antifungal peptides are amphipathic naturally and they are made up of both hydrophilic and hydrophobic domains (Tang *et al.*, 2018). The possible routes for the inhibition range from disintegration of cell membranes (binding to lipid bilayer structure and phospholipid groups) (Brogden, 2005; De Cesare *et al.*, 2020; Shai, 2002) to inhibit certain key pathways in the cell such as protein synthesis (Le *et al.*, 2017).

In the *in silico* analysis, peptides with antimicrobial peptides against gram-positive and gram-negative bacteria were identified from the fractionated trypsin and pepsin hydrolysates. Generally, peptides corresponding to different enzymatic hydrolysis exhibited different characteristics, in terms of their hydrophobic amino acid contents and the length of amino acid residues per peptide (Karami and Akbari-adergani, 2019). In our study, using the trypsin hydrolysis, the generated peptides were identified to PG-K111, Peptide 5, lactococcin Q and subtilosin A1 in the Antimicrobial Peptide Database (APD). PG-K111 is a neuropeptide from the tachykinin family and related to kassinin, which is involved in Kassina frog's neuropeptide signaling in a defense response (Grace *et al.*, 2001; Simmaco *et al.*, 1990). Tachykinin-related peptides were also known to have antimicrobial activity as reported in studies on *Triatoma infestans* hemolymph (Diniz

et al., 2020). Meanwhile, Peptide 5, a proline-rich peptide, was found to inhibit *Staphylococcus aureus* (Gram-positive bacteria) and *Klebsiella pneumoniae* (Gram-negative bacteria) (Dolashka *et al.*, 2011). Lactococcin Q, a bacteriocin, was originally demonstrated to inhibit *Lactococcus lactis* strains only (Zendo *et al.*, 2006). Later, the antimicrobial peptide was found to show high gene sequence cluster similarity to that of lactococcin G (Ishibashi *et al.*, 2015). Subtilosin A1 is an anionic bacteriocin that showed antibacterial activity towards certain gram-positive bacteria. Subtilosin A1 was an enhanced antimicrobial peptide by substituting the threonine with isoleucine at position 6 (Huang *et al.*, 2009). These antimicrobial peptides are believed to protect plants from invasive fungal and bacterial species. Their mode of action are believed to involve adhesion to microbial cells and penetrate the phospholipid membranes since the peptides are small in size (less than 10 kDa) and charge, and capable to alter hydrophobic and hydrophilic properties (Dicks *et al.*, 2018; Meade *et al.*, 2020). For example, studies on the mode of action by lactococcin G postulated that the peptide form a membrane-penetrating helix-helix structure which interacts with the membrane receptor of bacteria (Rogne *et al.*, 2008). Lactococci was also revealed to possess antifungal by producing novel antibiotic complex against food-borne pathogens, including fungi (Stoyanova *et al.*, 2010). Subtilosin is also found to inhibit various phytopathogenic fungi in a study on autochthonous *Bacilllus subtilis* isolated from *Prosopis juliflora* (Abdelmoteleb *et al.*, 2017).

The enzymatic hydrolysis of oil palm fruit mesocarps with pepsin yielded four peptides identified to *Plecoglossus altivelis* or aCATH, human drosomycin-like defensin, MiAMP2c and MiAMP2d. Initially aCATH was found to exhibit antimicrobial activity in fish (Lu *et al.*, 2011). A more recent studies found that cathelicidin peptides are effective against *Candida* species (Rapala-Kozik *et al.*, 2015; Scarsini *et al.*, 2015). Cathelicidins is generally thought to exhibit the antifungal activity through membrane destabilisation and internalisation (Ordonez *et al.*, 2014). Peptide acquired from our works also matched to human drosomycin-like defensin. Defensin is known for its antifungal properties (Lacerda *et al.*, 2014; Stotz *et al.*, 2009). The human drosomycin-like defensin from the APD matches was found to display fungal inhibition towards *Aspergillus* spp. and other clinically relevant filamentous fungi (Simon *et al.*, 2008). Similar to cathelicidins, drosomycin-like defensin, or defensin itself, interacts with the fungal target, either the cell membrane and/or the fungal cell wall (Struyfs *et al.*, 2021). Both vicilin-like macadamia antimicrobial peptides (MiAMP2c and MiAMP2d) were found to be inhibiting

various plant pathogenic fungi (Marcus *et al.*, 1999), though their inhibitory mechanism is still not clear.

Many antimicrobial peptides are known to exert antioxidant bioactivity as well (Memarpoor-Yazdi *et al.*, 2012). The presence of dipeptides and tripeptides in pepsin hydrolysate seemed to contribute to their antioxidant bioactivity as reported previously (Kawashima *et al.*, 1979; Samaraweera *et al.*, 2011; Wu *et al.*, 2020). The result of the *in silico* analysis was in agreement with their free radical-scavenging activity. Non-fractionated mesocarp pepsin hydrolysate showed a $DPPH_{SC}$ of 22.3%. The hydrophobicity of peptide is also considered as the key determinant in its antioxidant property, possibly due to the aromatic and sulphur containing structures that make them sequester radicals (Nwachukwu and Aluko, 2019; Zou *et al.*, 2016). The high amount of hydrophobic amino acid presence in the peptides confers to better antioxidant activity. However, this was not the case with pepsin hydrolysates as the identified peptides were found to have low quantity of hydrophobic amino acid residues. This suggests that there are novel antioxidant peptides present in the pepsin hydrolysate but was not being identified through the *in silico* analysis.

CONCLUSION

Confirmation of the presence of bioactive peptides from oil palm fruit mesocarps indirectly indicates that the oil palm by-product wastes such as pressed mesocarp fibres, generated after the oil extraction, could potentially serve as a promising source of bioactive peptides for a sustainable and eco-friendly management of the oil palm wastes. The conversion of these mesocarp wastes to peptides with antifungal bioactivities towards *G. boninense* also presents a more direct way to control the onset of basal stem rot disease caused by *G. boninense*. Results from this works also suggest that protein hydrolysates from oil palm fruit mesocarps could serve as valuable functional agents in healthy diets to manage metabolic diseases that arise from excessive levels of free radicals. However, *in vivo* antioxidant activity of these peptides and the mechanism underlying their protection of cellular oxidative stress protection need to be explored further. In the meantime, inhibition of *G. boninense* growth by higher concentration of the fractionated hydrolysates and the potential inhibitory or control mechanisms would also be established. These peptides could be utilised to develop novel agent with biologically active component in agriculture as natural and sustainable crop protection agents.

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